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Prevalence of *Fasciola spp*. in cattle in Al-Diwaniyah province

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Abstract

The genus *Fasciola*, sometimes known as liver flukes, is the source of the parasitic disease fasciolosis, which affects both humans and animals, the species *F. hepatica* and *F. gigantica* are the two species most frequently cited as the etiological agents of fasciolosis. The study was conducted to detect the prevalence of *fasciola* spp. in the typical abattoir in AL-Diwaniyah province from the period August 2022 to April 2023. The total number examined was 200 (112 male and 88 female). The study was all adult leaf-like parasites. The average parasite size body leight /body weight (56.4/7.84). Staining with alum carmine stain was used to identify Fasciola *spp.* and also note some changes on the affected livers by *Fasciola spp* such as cirrhosis and enlarged bile duct. The second part of the study was an epidemiological study and the effect of some factors on the prevalence such as age and sex. W here the number of infected cattle 18 in the rate of 9%. the highest infection was recorded in females compared to males (3) and females (15). The infected rate (2.67%) and (17.04%) male and females respectively also note the increasing number of infections with increasing age older than two years its (13.11) and the highest infection was recorded in January 40% followed by February and March

Keywords: Epidemiological, morphological, Liver flukes, Fasciola *spp*, cattle

Introduction

Fasciola is a member of the family Fasciolidae, which are huge, leaf-shaped parasites of animals, primarily herbivores, For hundreds of years, it has been recognized as a significant parasite of cattle and sheep, causing the liver of an animal to become infested with large, flat worms (1).There may be some modifications in the *Fasciola* life cycle, mostly within different final hosts, but the main effects that push the life cycle include the condition of suitable temperature and enough moisture (2). The life cycle of *Fasciola* spp. is complex, including different developmental stages and double hosts, an amphibious snail considered as the intermediate host and a mammalian final host (2,3). There are three different forms of clinical presentation (acute, subacute and chronic forms), (4). Infections with acute fasciolosis have been reported along the year as compared to the usual spring and autumn illnesses (5). Death that occurs suddenly without any warning symptoms sometimes anemia and appetite loss symptoms occure first, followed by death (6). Subacute fasciolosis often peaks between October and January.It is characterized by anemia, jaundice, and malaise and occurs on by eating a moderate amount of metacercaria (7). Fasciolosis is diagnosed based on clinical symptoms, grazing history, seasonal occurrence, laboratory testing of feces, and postmortem examination (8). This study aimed to study the epidemology of the infected cattle with *Fasciola spp*.in AL-Diwaniyah province.Identification and morphology of *Fasciola* spp.

Materials and Methods

Sample collection

This study was conducted on (200) slaughtered cattle (112 male and 88 female) collected from typical abattoir in AL-Diwaniyah three times a week and over the period from August ,2022 to end of April, 2023.

A. Macroscopic Examination

Following the collection of liver and bile duct from slaughtered cattle, the liver was carefully examined for any gross lesions using palpation, and numerous incisions to confirm the presence of *Fasciola* fluke in the liver parenchyma. The bile duct was then also checked for the presence of immature and mature flukes. When a cow was slautghtered, its liver were carefully examined alongside. The livers and bile ducts were first checked for the presence of *Fasciola spp*. The flucks collected With the aid of forceps, the bile ducts were longitudinally incised into the gall bladder, and the parasites were extracted while taking all required precautions to prevent any harm to the parasite. Each parasite was completely cleaned individually 2 to 3 times in a 0.9% saline solution to eliminate debris and contamination after the infected livers were manually pressed to macerate the parenchyma and the flukes were carefully removed. The samples were then transported to the lab and kept at 4°C in 70% ethanol. If any *Fasciola* flukes were identified as immature and mature worms, and gross lesions were noted (9).

B. Staining procedure of adult *Fasciola*.

Two flukes from each group were prepared for alum carmine staining after the flukes had died. The flukes were properly cleaned with 0.1M phosphate buffered saline, pH 7.4, placed between two slides, tied on both sides with rubber bands, and then submerged in 10% formalin for at least 12 hours to facilitate fixing. The formalin was then removed by washing them in successively tap water. The washed flukes were dehydrated three times with progressively stronger

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alcohol before being overnight dyed with acetic alum carmine stain. The flukes were cleaned in xylene, clarified with 1% acid alcohol, dehydrated with graded series of ethanol, washed in ammonia water, and mounted with DPX. (10)

Ethical approval

The local Committee for Animal Care and Use at the College of Veterinary Medicine, University of Al-Qadisiyah , Al-Qadisiyah ,Iraq, reviewed and approved all procedures. involved in the current study.

Statistical Analysis

The Statistical Analysis System program was applied to detect the effect of sex, age and months on the *fasciola spp* .infection in cattle .Chi-square test was used and P value less than 0.05 was significat (SAS 2012) .

Results

1-Macroscopic study

Grossly, the livers of infected calves displayed cirrhosis, paleness in the hepatic lobes, and thickness in the bile ducts, along with the presence of an adult parasite that looked like a leaf. In contrast to the brown smooth healthy liver, the infected liver seems to be harder and has an uneven surface due to parenchymal congestion and the creation of numerous abscesses, which appear as pale necrotic regions with fibrosis as refers to figure (1).

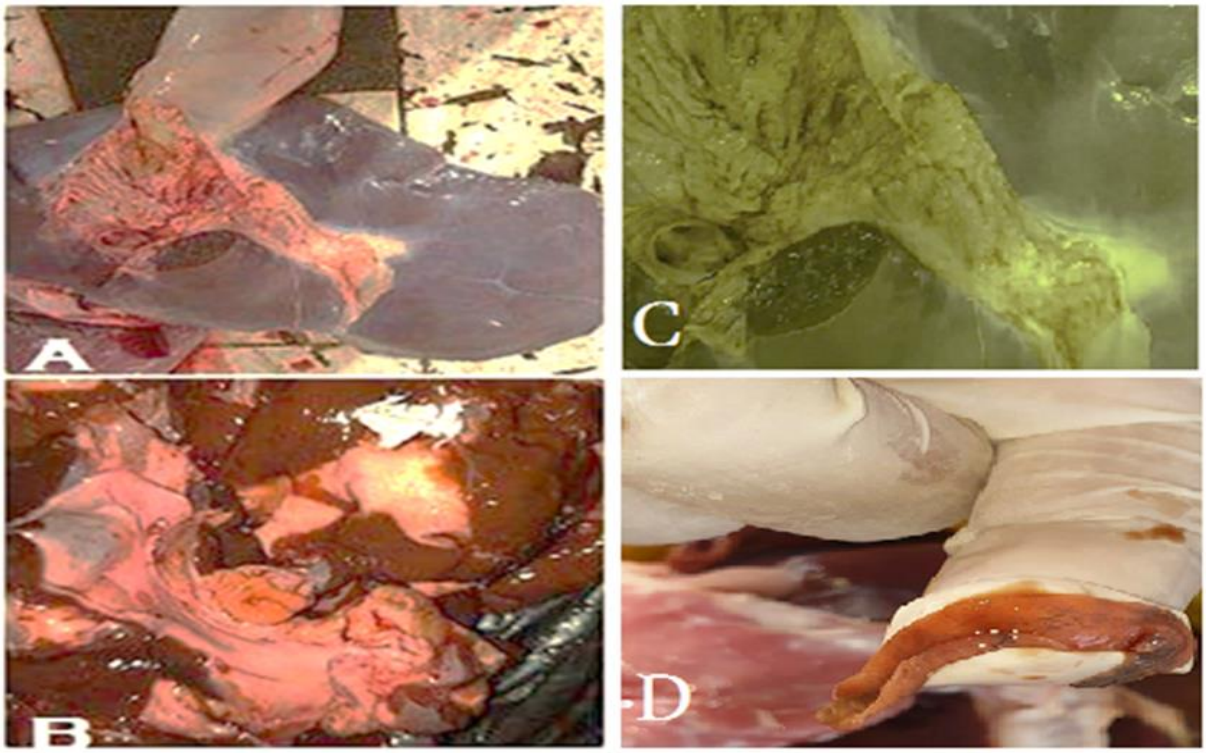

Figure 1: gross portion of a cow's liver that is infected. *A and B infected liver of cattle with normal bile duct, C. Liver of cattle infected with fasciola spp and show calcification and hemorrhage, C.Infected liver show adult fasciola spp on the liver of cattle and D. Adult fasciola spp*

2-Morphological examination:

Adult *Fasciola* worms made up the majority of those taken from infected cattle livers. The adult liver flukes had a flattened shape, grayish brown in color, and a leaf-like appearance. Taking into account the index of BL/BW measurements. *Fasciola* samples were measured macroscopically and twenty –eight samples were taken to find the average length and width it was found that mean length 4.56mm and mean wight 7.48mm **show** Figure (2)

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Figure 2: Adult *Fasciola gigantica* size measure approximately 75 by 9mm

3. Microscopic examination

The worms used in this investigation were isolated and stained with alum carmine. The cephalic cone and two shoulders on the anterior end are wide, and the borders are converging. The anterior end of a cone-shaped protrusion is where the little, anterior oral sucker is located. At the front end lies the acetabulum, which has a bigger sucker than the oral sucker. Worm with a smooth tegument covering it; the posterior end points back as seen Figures (3and 4).

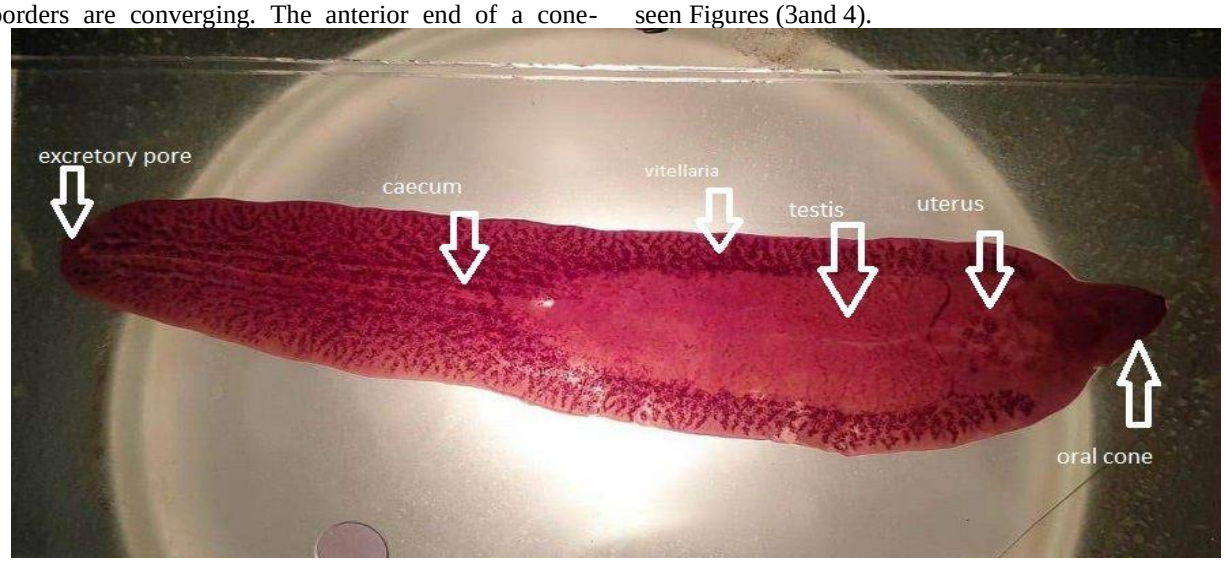

Figure 3: Adult *Fasciola spp* under microscope (40X objected lens)

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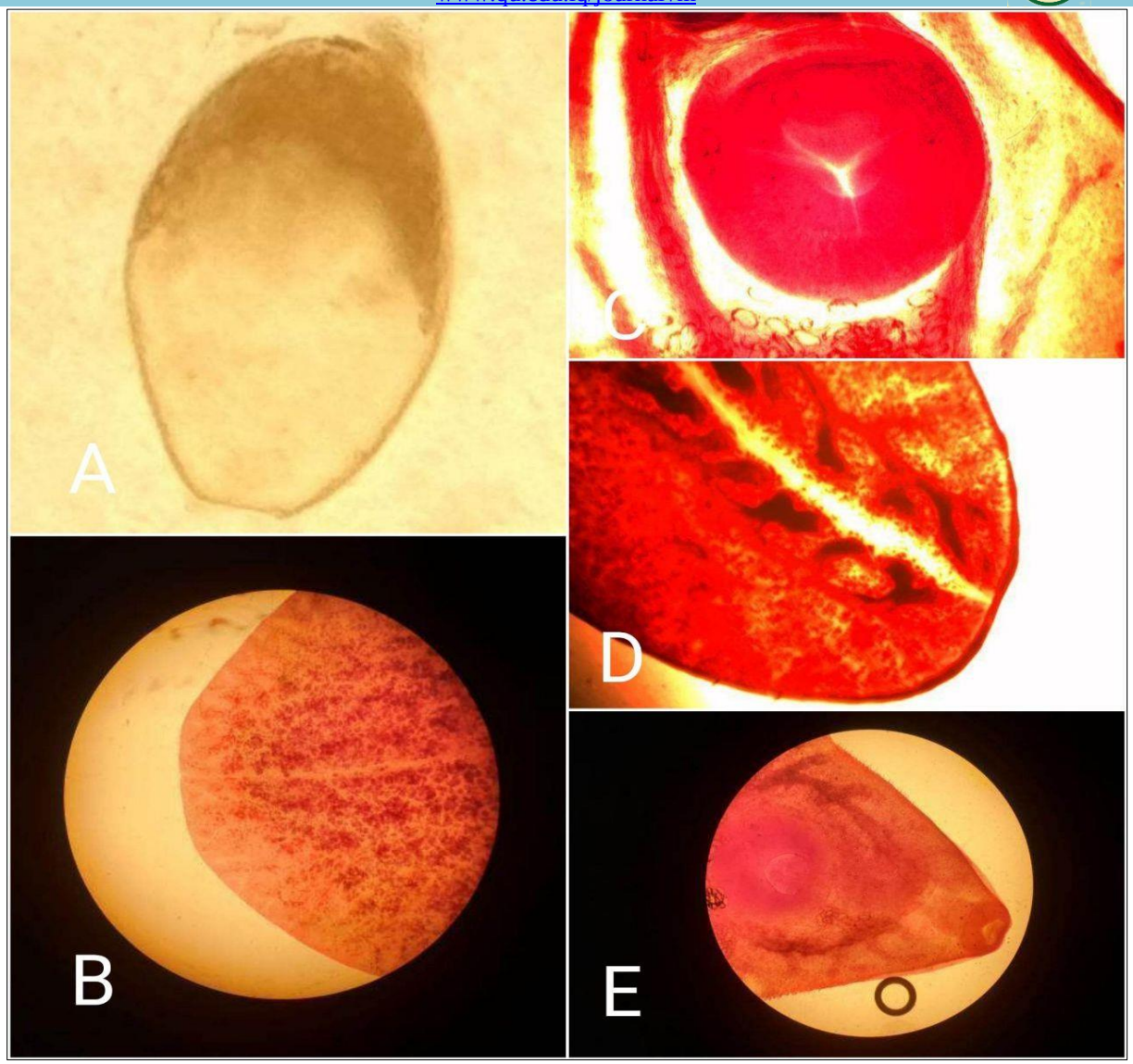

Figure 4: *Fasciola spp* after staining and measures by light microscope (40X) A) Egg B) Posterior end with intestinal branches, C) Ventral suckers, D) Posterior end with intestinal branches,E)Oral suckers

4. The Prevalence of *Fasciola* infection

The overall rates of cow's fascioliasis from August 2022 to end of March 2023 at different location of Al-Diwanyiah 9٪(18/200) .

4.1. The infection rates according to sex factor

Male recorded 2.67 ٪ (3/112) rate of infection while female recorded 17.04 ٪(15/88) table (2).

Table (2): The infected rate of *Fasciola* spp according to sex group

Sex	No. of sample examined	No. of infection	Percentage	Chi-square value	P
Males	112	3	2.67	12.42	<0.000.1(S)
Females	88	15	17.04		

S=significant difference at P<0.05

4.2. The infection rates according to age group:

The study showed that the highest infection rate was recorded at >2 years age group 13.11٪ (16\122).

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While the lowest infection rate recorded at ≤ 2years age 2.56٪ (2/78) rate of infecation with significant differences P (0,001) as shown table (3)

Table 3: Infection rates according to age group

Age group	No.of samples	No.of infected	Percentage ٪	Chi-square value	P
≤2 year	78	2	2.56	6.46	0,001(s)
>2 years	122	16	1311		

S=significant difference at P<0.05

4.3. The infection rate of *Fasciola* spp. according to months of study:

The highest rate of cattle fascioliasis infection was seen in January 40٪(10/25) followed by February and March the infection rate 16 ٪(4/25). While lowest infection rate was recorded in December, November, October, September and August 0 ٪(0/25) Table (4)

Table 4: The infection rate for *Fasciola spp* according to months of research.

Month	No.of sample	No.of infection	Percentage ٪	Chi-square value	P value
August	25	0	0	4566	<0.0001(s)
September	25	0	0		
October	25	0	0		
November	25	0	0		
December	25	0	0		
January	25	10	40		
February	25	4	16		
March	25	4	16		

S=significant difference at P<0.05

Discussion

1.Prevalence study.

Although, the scarcity of studying *Fasciola* in livestock within the Diwaniyah governorate and evaluating it according to the risk variables, it is clear to us that the infection rate is generally low, estimated at 9% (18/200). And it was consistent with what was shown by previous studies in and for several hosts summarized from many studies (11-16) .The study identified factors of month, age and sex to show the effects and variation in infection with liver flukes if the female gender had a significant difference from males and the older ages of more than two years are higher than the young ages and for reasons summarized in the effective exposure to infection with permanent grazing and the level of immunity and tissue resistance to the parasite (17,18). Seasonally, the study found that the infection was concentrated within the last three months of the study, January - March. It wasn't consistent with the study of (19).

2. Gross study:

The liver swelled in response to acute inflammation as the wandering juvenile fluke mechanically damaged the parenchyma, which was one of the macroscopic alterations seen in the chronically infected livers (20). The liver is also described as being uneven, solid, edematous, with hemorrhagic channels, and adherent at the sliced surface. The current study's chronic instances also demonstrated that the livers were hard, tough, and the cut section had signs of calcification, while the affected ducts had cholangitis and adult fluke were swollen, distended, thickened, and affected by calcification. These were attributed to the immunological response of infiltrating macrophages and lymphocytes, which combined with the fibrotic repair of the necrotic sites during the advanced fasciolosis stage. Inflammatory biliary conditions such hyperplastic cholangitis, ductular wall thickening, and intra- and extrahepatic biliary dilatation are brought on by the adult flukes roaming in the biliary tract over time (21). The variations in calcification may result from species variation, suggesting a possible direct connection to host resistance. Additionally, it's likely that the calcification in cattle affects fluke eating behavior, and this has an impact on the extent of the calcification.

3. Microscopic Study:

According to the study's findings, adult worms have flattened, grayish brown bodies that resemble leaves (23). In the current investigation, the discovered worm isolates were categorized as being similar to *F. hepatica*, with body lengths ranging from 35 to 52 mm and widths

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between 7 and 10 mm. Results that are in accordance with (24). That the majority of liver flukes are hermaphrodites, with a flattened dorsoventral body, a blind alimentary tract, and suckers for attachment. The mature Fasciola hepatica is located in the bile ducts. The current findings are consistent with other literatures (25-27) that addressed comparable work using the same criteria as those used in the study.The claims made by (26). That host species, age, and immune responses because of a potential prior infection can affect external measurements of the differences in body length, width, cone length, and width of *fasciola* may help

conclusions

The study measured the overall prevalence fascioliasis in the cattle that were see 9% (18/200) no a discernible difference between the sexes (male 2.67% (3/110), female 17,04(15/88), and the study recorded an increase in the number of infection with increasing age, and increasing infection through January and lowest in February and March. The results of the gross examination and the morphological examination to differentiate between the types of *Fasciola* are insufficient to distinguish between them.

Conflict of Interest: The authors declare that there is no conflict of interest

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